Nucleosides. XV. Decarboxylative Elimination of 2′-Deoxynucleoside Uronic Acids[1a,b]

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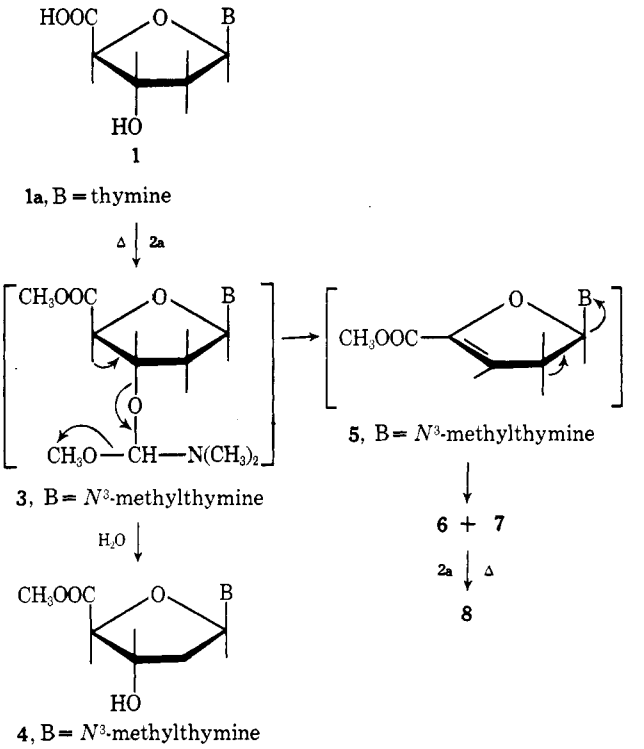
Abstract: A practical conversion of 2′-deoxynucleoside-5′-carboxylic acids (**1**) to pyrimidine and purine 2,3-dihydrofuryl derivatives (**12**) *via* a single step "decarboxylative-elimination" reaction is described. Thus treatment of **1** with dimethylformamide dineopentyl acetal (**2b**) in DMF at 80–90° gives **12** in high yield. Two possible pathways are described for the transformation which comprises a useful, selective degradation of C-5′ from a 2′-deoxynucleoside. Catalytic hydrogenation of **12** gives the corresponding tetrahydro-2-furyl derivatives (**13**) of the *R* configuration, which are of biochemical interest. Substitution of dimethylformamide dimethyl acetal (**2a**) in the reaction with thymidine-5′-carboxylic acid (**1a**) leads to methyl 3-methylthymidine-5′-carboxylate (**4**) as the principal product (29% yield) along with some 1,3-dimethylthymine (17% yield). The latter arises as a consequence of consecutive base-catalyzed elimination reactions on methyl thymidine-5′-carboxylate followed by alkylation of intermediate 3-methylthymidine. The nmr spectra of **12** and **13** are discussed.

The 1,1-dialkoxydimethylaminomethanes (**2**, dimethylformamide dimethyl acetals) are versatile reagents that have found considerable application in the elucidation of the chemistry of nucleosides and nucleotides. The reactions of **2** include both the inter-[2-4] (*vide infra*) and intramolecular[5] alkylation of nucleic acid components, the protection of an exocyclic amino group of, for example, a cytosine, adenine, or guanine moiety,[4,6,7] promotion of cyclic phosphate formation,[4] cleavage of specific internucleotide bonds,[3] and the dephosphorylation of nucleotides.[8] The utility of this unique class of reagents has now been extended to the conversion of 2′-deoxynucleoside-5′-carboxylic acids (**1**) to pyrimidine and purine 2,3-dihydrofuryl derivatives (**12**) *via* a single step "decarboxylative-elimination" reaction.

Esterification as well as alkylation was achieved on treatment of 1-(2-deoxy-β-D-*erythro*-pentofuranosyl-5-uronic acid)thymine[9] (**1a**, thymidine-5′-carboxylic acid) with dimethylformamide dimethyl acetal (**2a**) in DMF at 100°, the reaction affording methyl 3-methylthymidine-5′-carboxylate (**4**) as the principal product (29% yield) along with 17% of 1,3-dimethylthymine (**8**) (Scheme I). The same ester (**4**) is obtained (87% yield) from the action of diazomethane on **1a**. The isolation of **8** was, however, surprising in view of the fact that corresponding products had not been detected in alkylation of pyrimidine and purine nucleosides with dimethylformamide dimethyl acetal.[2] Thus, the methylation of 5-fluoro-2′-deoxyuridine (**9a**) with **2a** gives the 3-methyl derivative (**9b**) in 81% yield as the only product.

Scheme I



$(CH_3)_2NCH(OR)_2$

2

2a, R = CH_3
b, R = $(CH_3)_3CCH_2$

A recent report[10] from this laboratory described the facile conversion of methyl 3-methyl-3′-*O*-(methylsulfonyl)thymidine-5′-carboxylate to methyl 1-(2,3-dideoxy-3,4-didehydro-β-D-ribofuranosyl-5-uronate)-3-

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(1) (a) This investigation was supported in part by U. S. Public Health Service Research Grant No. FR-5529 from the National Cancer Institute and in part by an institutional grant to the Detroit Institute of Cancer Research, Division of the Michigan Cancer Foundation, from the United Foundation of Greater Detroit. (b) Presented in part at the 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1970, Abstract CARB-3.

(2) J. Žemlička, *Collect. Czech. Chem. Commun.*, **35**, 3572 (1970).

(3) A. Holý and J. Žemlička, *ibid.*, **34**, 3921 (1969).

(4) A. Holý, S. Chládek, and J. Žemlička, *ibid.*, **34**, 253 (1969).

(5) J. Žemlička, *ibid.*, **33**, 3796 (1968).

(6) J. Žemlička and A. Holý, *ibid.*, **32**, 3159 (1967).

(7) J. Žemlička, S. Chládek, A. Holý, and J. Smrt, *ibid.*, **31**, 3198 (1966).

(8) J. Žemlička and S. Chládek, *Tetrahedron Lett.*, **9**, 715 (1969).

(9) G. P. Moss, C. B. Reese, K. Schofield, R. Shapiro, and A. R. Todd, *J. Chem. Soc.*, 1149 (1963).

(10) J. Žemlička, R. Gasser, and J. P. Horwitz, *J. Amer. Chem. Soc.*, **92**, 4744 (1970).

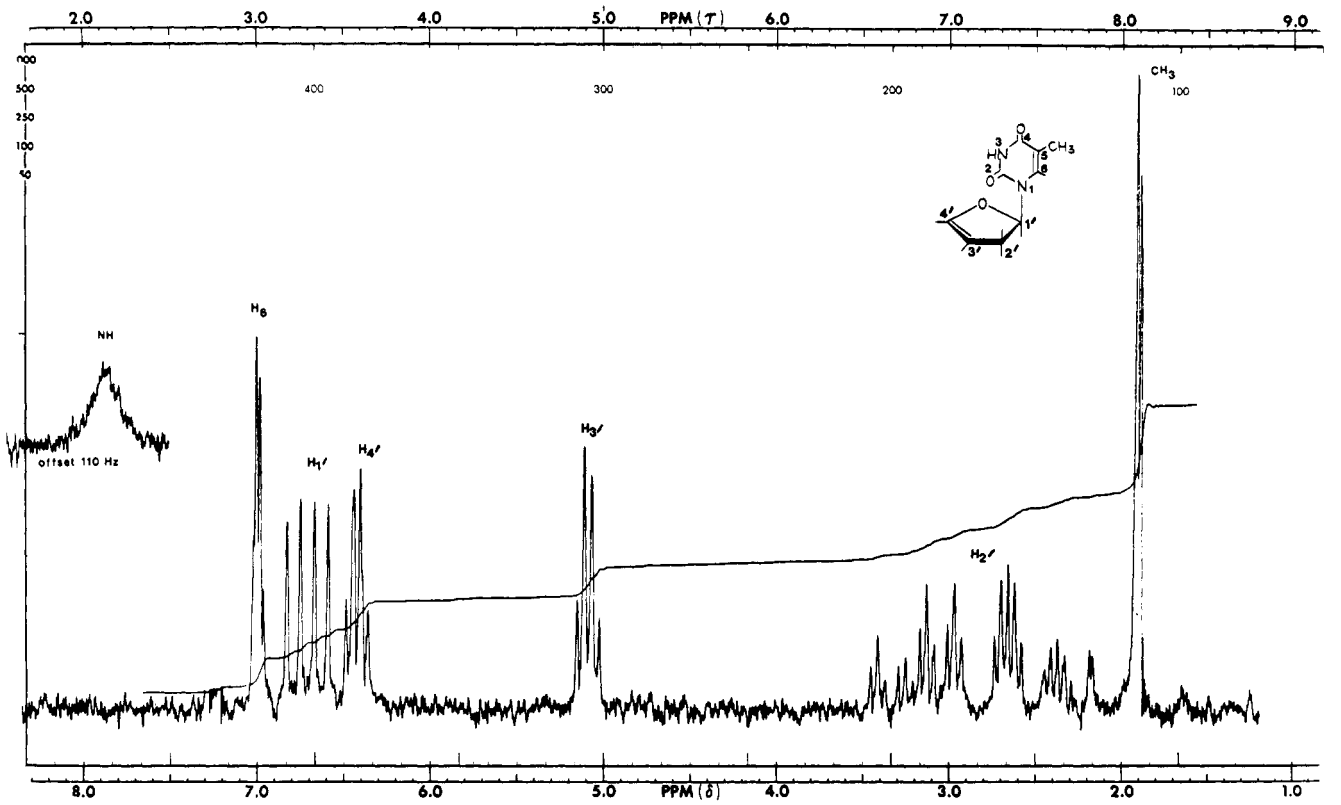
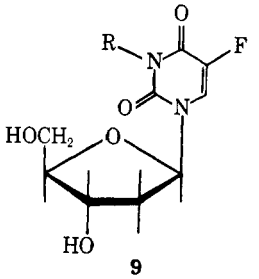


Figure 1. Nuclear magnetic resonance spectrum of 1-(2′,3′-dideoxy-3′,4′-didehydro-β-D-erythrofuranosyl)thymine (**12a**) ($CDCl_3$, TMS as internal standard).

methylthymine by the action of triethylamine in DMF at 100°. The observations suggested a reaction path to **8** through a series of transformations, the first of which requires esterification and transacetalation of **2a** to give



9
9a, R = H
9b, R = CH_3

the 3′-amidoacetal ester (**3**). Consecutive base-catalyzed elimination reactions proceeding from **3** would lead to 3-methylthymine (**6**) and methyl furoate (**7**) *via* **5**. Finally, methylation of **6** with excess **2a** would explain the detection of **8**.

The suggestion of an amidoacetal function as an effective leaving group in the initial elimination reaction, which is the key reaction in the sequence, is based on the observation[5] that intramolecular displacement of a 5′-amidoacetal grouping from xanthosine provides a practical synthesis of N^3,5′-cycloxanthosine.

In an attempt to gain additional support for the proposed pathway of elimination, dimethylformamide dineopentyl acetal (**2b**) was substituted for **2a** to avoid possible competitive effects arising as a consequence of alkylation and/or esterification (*vide supra*). Treatment of thymidine-5′-carboxylic acid[9] (**1a**) with **2b** in DMF at 80–90° for 4.5 hr gave a product (80% yield) with properties consistent with 1-(2,3-dideoxy-3,4-didehydro-β-D-erythrofuranosyl)thymine[11] (**12a**). Moreover, the same reaction applied to the 5′-carboxylic acids of 2′-deoxyuridine[12] (**1b**), 5-fluoro-2′-deoxyuridine[13] (**1c**), and 2′-deoxyadenosine (**1d**) gave the corresponding 2,3-dihydrofuran derivatives (**12b**, **12c**, and **12d**, respectively) in good yield.

The unexpected decarboxylative sequence leading to **12** shares, in common with the suggested pathway to **4**, a *sine qua non* intervention of a 3′-amidoacetal intermediate **10**. A difference between the two reactions develops as a consequence of decarboxylation which probably occurs in concert with the elimination of the amidoacetal group (Scheme II). A less attractive alternative is the loss of carbon dioxide from **11** subsequent to elimination.

Further studies are currently in progress to elaborate the scope and limitations of the transformation. It is, however, apparent that the combined steps of oxidation and decarboxylative elimination constitute a selective and thereby a potentially useful degradation of C-5′ from a 2′-deoxy-*erythro*-nucleoside.

The olefinic derivatives (**12**), which may be regarded as the simplest members of a recently described[10,14,15]

(11) Application of systematic nomenclature to **12a** leads to the name (−)-(*R*)-2-(thymin-1-yl)-2,3-dihydrofuran.

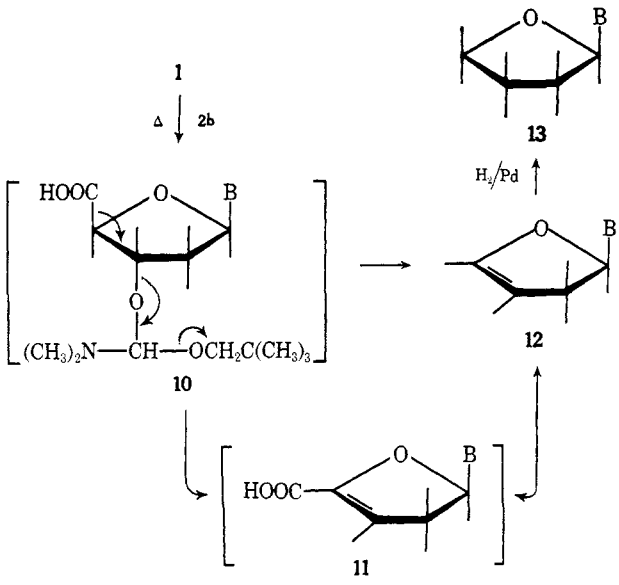
(12) K. Imai and M. Honjo, *Chem. Pharm. Bull.*, **13**, 7 (1965).

(13) K. C. Tsou, N. J. Santora, and E. E. Miller, *J. Med. Chem.*, **12**, 173 (1969).

(14) G. H. Jones and J. G. Moffatt, 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, Abstract CARB-15; U. S. Patent 3,457,255 (1969); *Chem. Abstr.*, **72**, 3727 (1970).

(15) G. Kowollik, K. Gaertner, and P. Langen, *Tetrahedron Lett.*, 1737 (1971).

Scheme II



a, B = thymine c, B = 5-fluorouracil
b, B = uracil d, B = adenine

class of 2′,3′-dideoxy-3′,4′-didehydronucleosides, exhibit in common with the latter high, negative values of optical rotation.[10,15] The nmr spectra of **12** (*cf.* Figure 1) are characterized by a pair of quartets centered at *ca.* δ 5.1 and 6.4 which are assigned to $H_{3'}$ and $H_{4'}$, respectively, by analogy with the olefinic protons of dihydrofuran[16] itself. These findings coupled with the absence from the same spectra of peaks assignable to methylene protons adjacent to a cyclic ether linkage preclude the possibility of a 2,5- or a 4,5-dihydrofuran structure for **12**. The $H_{1'}$ proton of **12** appears as a multiplet of four and comprises the X portion of an ABX system. The $H_{2'}$ protons of the methylene group (AB portion of ABX system) form two sets of multiplets belonging each to one proton. These data lend further persuasion to the assigned structure (**12**).

Several years ago Robins and Robins[17] showed that one may assign the anomeric configuration to purine 2′-deoxyribofuranosyl nucleosides with reasonable confidence on the basis of the nmr absorption band of $H_{1'}$. Thus, the β anomers are characterized by a "pseudo-triplet" for $H_{1'}$ with an apparent coupling constant of $J_{H_{1'}} \cong 7$ Hz and a peak width ≅ 14 Hz. By contrast, the α anomers show a multiplet of four with coupling constants $J_{H_{1'}} \cong 3$ and 7 Hz and peak width ≅ 10 Hz. These criteria for anomeric assignment appeared to be general for 2′-deoxy-D-ribofuranosyl nucleosides irrespective of the heterocyclic base.[18-20]

The $H_{1'}$ proton of **12** resembles in multiplicity (*cf.* Table I) the $H_{1'}$ of 2′-deoxy-α-D-ribofuranosyl nucleosides. On the other hand, the apparent coupling constants and peak widths of the "glycosyl" proton in the two cases differ significantly. Accordingly, the observed similarities were simply ascribed to coincidence since the presence of unsaturation in **12** would be expected to alter greatly the conformation of the dihydrofuryl moiety relative to a 2′-deoxyribofuranosyl residue and would thereby vitiate the comparison. However, Goodman and coworkers have noted that the patterns for the anomeric protons of methyl 2,3-dideoxy-3-acetamino-5-*O*-trityl-β-D-ribofuranoside,[21] 2′,3′-dideoxy-3′-aminoadenosine,[22] 3′,5′-bis-*O*-(*p*-toluoyl)thymidine, and 2′-deoxy-5-(trifluoromethyl)uridine[23] were all actually quartets and as such appeared to constitute exceptions to the general rule of assignment of anomeric configuration.

Table I. Nuclear Magnetic Resonance Splitting Patterns for the $H_{1'}$ Proton of Starting Materials and Reaction Products

Compd	Multiplicity	Signal width, Hz	δ[a]	$J_{1',2'}$ and $J_{1',2''}$, Hz
1a	q	15	6.40 (a)	9.0, 6.0
1b	q	15	6.32 (a)	10.0, 6.0
4	q	14	6.57 (b)	9.0, 5.0
4	t[b]	14.5	6.42 (a)	8.0, 6.5
12a	q	14	6.73 (b)	9.5, 4.5
12b	q	14	6.78 (b)	9.5, 4.5
12c	m[c]	14	6.70 (b)	10.0, 4.5
12d	q	14	6.78 (c)	7.0, 6.0
13a	q	9–10	6.00 (b)	5.0, 4.0
13d	t	9	6.00 (b)	4.0, 5.0

[a] Solvents used are (a) DMSO-d_6, (b) $CDCl_3$, and (c) acetone-d_6. [b] Analysis (*cf.* Figure 2) has shown this signal to be more likely a quartet (as in **12d**) whose two middle peaks are not well resolved. [c] Signal appears as a quartet which is further split by a long-range coupling with fluorine. A similar situation was reported earlier for $H_{1'}$ proton of 5′-fluorouracil nucleosides: R. J. Cushley, T. Wempen, and J. J. Fox, *J. Amer. Chem. Soc.*, **90**, 709 (1968).

In the course of the present study, we observed that the anomeric protons of the carboxylic acids **1a**,**1b**, and of methyl 3-*N*-methylthymidine-5′-carboxylate (**4**), also gave rise to an unevenly spaced multiplet of four. Measurements in $CDCl_3$ and DMSO-d_6 have shown in the case of **4** that the pattern (Figure 2) is solvent dependent, which further suggests that the differences in the values of $J_{1',2'}$ and $J_{1',2''}$ for **12a–c** relative to **12d** more likely reflect the change of the solvent than the change of heterocyclic base (from a pyrimidine to a purine). It is these several observations that prompt the suggestion of a note of caution in the assignment of configuration of 2′-deoxyribofuranosyl nucleosides on the basis of nmr patterns for $H_{1'}$ in situations wherein only one anomer is available for such measurements.

Catalytic (Pd/C) hydrogenation of **12** gave the 2′,3′-dideoxyerythrofuranosyl (tetrahydro-2-furyl) derivatives (**13**) which may be viewed as analogs of deoxynucleosides. The nmr spectra of **13a**,**c**,**d** are consistent with the proposed structures. The $H_{1'}$ protons in the latter appear at a considerably higher field than those of unsaturated derivatives **12a–d**. The 2′,3′-dideoxyerythrofuranosyl derivatives show a peak width of 9–10 Hz for $H_{1'}$ compared to values of 14–15 Hz noted for **12a–d** and as well for **1a**,**b** and **4**. Whereas $H_{1'}$ in **13d** appears as a triplet, the same proton in **13a** is present as an incompletely resolved quartet with values of 5 and 4 Hz, respectively, for $J_{1',2'}$ and $J_{1',2''}$. The $H_{2'}$ and $H_{3'}$ methylene groups both form multiplets at

(16) L. M. Jackman, "Nuclear Magnetic Resonance Spectroscopy," Pergamon Press, New York, N. Y., 1959, pp 62, 88.
(17) M. J. Robins and R. K. Robins, *J. Amer. Chem. Soc.*, **87**, 4934 (1965).
(18) R. U. Lemieux, *Can. J. Chem.*, **39**, 116 (1961).
(19) M. J. Robins and R. K. Robins, *J. Org. Chem.*, **34**, 2160 (1969).
(20) M. J. Robins, T. A. Khwaja, and R. K. Robins, *ibid.*, **35**, 636 (1970).
(21) C. A. Anderson, W. W. Lee, L. Goodman, and B. R. Baker, *J. Amer. Chem. Soc.*, **83**, 1900 (1961).
(22) W. W. Lee, A. Benitez, C. A. Anderson, L. Goodman, and B. R. Baker, *ibid.*, **83**, 1906 (1961).
(23) K. J. Ryan, E. M. Acton, and L. Goodman, *J. Org. Chem.*, **31**, 1181 (1966).

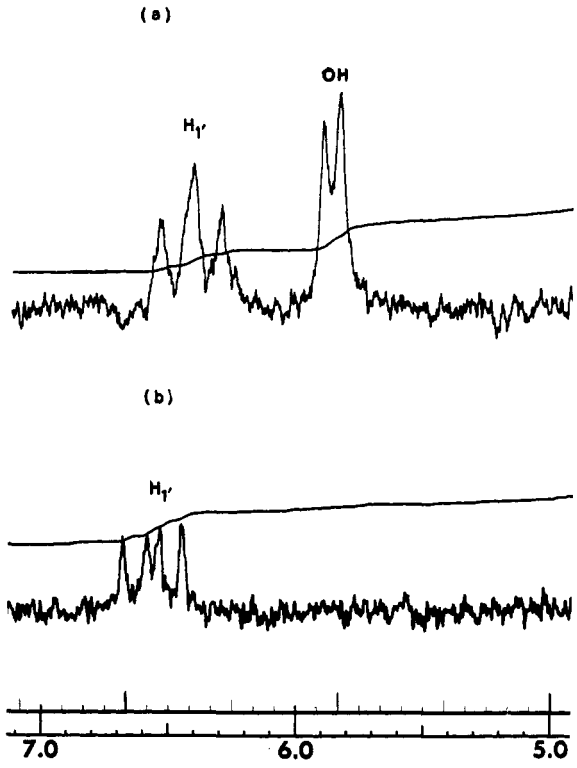


Figure 2. Nuclear magnetic resonance spectrum (anomeric proton region) of methyl *N*-methylthymidine-5′-carboxylate (**4**): (a) in DMSO-d_6 with DSS as internal standard, (b) in $CDCl_3$ with TMS as internal standard.

δ 2.1–2.3 whereas the $H_{4'}$ methylene group gives rise to a quartet positioned, as expected, at an appreciably lower field (about δ 4.1).

Several 6-substituted 9-(tetrahydro-2-furyl)purines have been obtained[24–26] as racemic mixtures from the acid-catalyzed addition of 2,3-dihydrofuran to the appropriate purine. The products have shown significant antitumor activity toward a variety of experimental mouse tumors.[26] The synthesis of **13**, described in the present work, leads to pyrimidine and purine 2-tetrahydrofuryl derivatives of the *R* configuration which are, therefore, of considerable biochemical interest.[26a]

Experimental Section

General Procedures. Evaporations were carried out in a Büchi rotary evaporator *in vacuo* at a bath temperature below 40° unless stated otherwise. Melting points were determined on a Thomas-Hoover apparatus (capillary method) and are uncorrected. Samples for analysis were dried at 10^{-3} mm over P_2O_5 at room temperature for 8 hr unless stated otherwise. Microanalyses were performed by Micro-Tech Laboratories, Inc., Skokie, Ill. Thin layer chromatography (tlc) was performed on 6 × 2-cm, precoated, silica gel F-254 aluminum foils (Merck, Darmstadt, Germany) in solvents S_1 (chloroform–methanol, 95:5), S_2 (chloroform–methanol, 9:1), and S_3 (chloroform–methanol, 4:1). Preparative tlc was performed on 2-mm thick, 20 × 20 cm loose layers of silica gel (70–325 mesh ASTM, Merck, Darmstadt, Germany) containing 1% of fluorescent indicator (Leuchtpigment ZS Super, Riedel-De Haën, Hannover, Germany). Tlc in solvent S_4 (2-propanol–concentrated ammonium hydroxide–water, 7:1:2) was performed on glass plates coated with Avicel microcrystalline cellulose (Brinkmann Instruments, Inc., Westbury, N. Y.). The layers were prepared by covering the glass plates (16, 6 × 2 cm each) with a suspension of Avicel (6 g) and the above fluorescent indicator (60 mg) in ethanol (15 ml) and air-dried at room temperature overnight. Paper electrophoresis was conducted on a Savant electrophoresis flat plate, using 0.02 *M* disodium hydrogen phosphate (pH 7.5) as a buffer on Whatman No. 1 paper at 40 V/cm for 1 hr. Uv-absorbing compounds were detected using a Mineralight lamp; unsaturated substances and those having uracil or thymine nuclei were also detected with potassium permanganate spray. Optical rotations were determined with a Perkin-Elmer Model 141 polarimeter. Uv absorption spectra were obtained by using a Cary recording spectrophotometer. The ir spectra were measured in a Perkin-Elmer Model 21 spectrometer. Nmr spectra were obtained using a Varian A-60A spectrometer; TMS was used as internal standard with $CDCl_3$ and acetone-d_6, DSS with DMSO-d_6. Dimethylformamide was dried with Linde Molecular Sieves, 4A. Petroleum ether was of a 30–60° boiling point range. Dimethylformamide dimethyl and dineopentyl acetals were products of Aldrich, Milwaukee, Wis.

Thymidine-5′-carboxylic Acid (1a). The platinum-catalyzed oxidation of thymidine followed the procedure of Moss, *et al.*,[9] to give **1a** in 65% yield: mp 258–260° dec (lit.[9] 263–265°); $[\alpha]^{23}D$ +9.4°, $[\alpha]^{23}_{436}$ +22.4°, $[\alpha]^{23}_{365}$ +42.6° (*c* 0.5, H_2O); uv max (H_2O) 268 nm (ϵ 8700), min (ϵ 2300); nmr (DMSO-d_6) δ 8.08 (s, 1, H_6), 6.36 (q, 1, $H_{1'}$), 1.82 (s, 3, CH_3).

2′-Deoxyuridine-5′-carboxylic Acid (1b). The same oxidation procedure applied to 2′-deoxyuridine gave **1b** in 67% yield, mp 222–225° dec (lit.[12] 222–223° dec). However, tlc (S_4) and an nmr spectrum (*vide infra*) indicated traces of starting material. Recrystallization of the product from water raised the melting point to 234–235° dec and **1b** afforded a uv spectrum consistent with the literature:[12] $[\alpha]^{24}D$ +18.6°, $[\alpha]^{24}_{436}$ +45.4°, $[\alpha]^{24}_{365}$ +91° (*c* 0.5, H_2O); nmr (DMSO-d_6 + D_2O) δ 8.17 (d, 1, H_6), 6.33 (q, 1, $H_{1'}$), 5.74 (d, 1, H_5).

5-Fluoro-2′-deoxyuridine-5′-carboxylic Acid (1c). Application of the method described by Tsou and coworkers[13] gave **1c** in 60% yield, mp 224–225° (lit.[13] 224–225°), and all other physical constants in accord with recorded values. In addition, the purity of **1c** as well as the other starting acids (**1a–d**) was checked by tlc (S_4) and paper electrophoresis.

2′-Deoxyadenosine-5′-carboxylic Acid (1d).[27,28] To a solution of 2′-deoxyadenosine (1.005 g, 4 mmol) in water (500 ml) were added dropwise with stirring at room temperature aqueous solutions of potassium permanganate (400 ml, 2.52 g, 16 mmol) and potassium hydroxide (50 ml, 0.79, 12 mmol). After stirring for 2 days, excess permanganate was destroyed with 30% hydrogen peroxide, the insoluble portion was removed by filtration, and the filter cake was washed with water. The filtrate was evaporated to *ca.* 30 ml and the solution was acidified to pH 4.5 with hydrochloric acid. The precipitate **1d** was collected and dried; yield 0.3 g (28%), mp 248–250°. Recrystallization from water raised the melting point to 255° (lit.[28] 257°); uv max (0.001 *N* NaOH) 260 nm (ϵ 12,400), min 228 (ϵ 2600); nmr (DMSO-d_6) δ 8.44 (s, 1, H_8), 8.17 (s, 1, H_2), 7.26 (broad s, 2, NH_2), 6.53 (t or q, poorly resolved, $H_{1'}$).

1-(2′,3′-Dideoxy-3′,4′-didehydro-β-D-erythrofuranosyl)thymine (12a). Thymidine-5′-carboxylic acid[9] (**1a**, 0.48 g, 1.87 mmol) and dimethylformamide dineopentyl acetal (**2b**, 1.388 g, 6 mmol) in dimethylformamide (20 ml) were heated at 80–90° for 4.5 hr. After cooling, the reaction mixture was evaporated at 0.1 mm and 40° (bath temperature); the syrupy residue was dissolved in water (5 ml) and extracted twice with methylene chloride or chloroform (10 ml each). The organic layer was dried ($MgSO_4$) and evaporated

(24) R. K. Robins, E. F. Godefroi, E. C. Taylor, L. R. Lewis, and A. Jackson, *J. Amer. Chem. Soc.*, **83**, 2574 (1961).

(25) L. R. Lewis, F. H. Schneider, and R. K. Robins, *J. Org. Chem.*, **26**, 3837 (1961).

(26) W. A. Bowles, F. H. Schneider, L. R. Lewis, and R.K. Robins, *J. Med. Chem.*, **6**, 471 (1963).

(26a) Note Added in Proof. After this paper had been submitted to press, we became aware of the work of S. Hillers, R. A. Zhuk, and M. Lidaks, *Dokl. Akad. Nauk. SSSR*, **176**, 332 (1967), who described racemic forms of compounds **13a** and **13c**, mp 176–178° and 164–165°, respectively.

(27) Compound **1d** was prepared earlier by oxidation of 2′-deoxyadenosine with chromium trioxide in pyridine in 14% yield.[28a] Using potassium permanganate we have circumvented the elaborate steps of preparative paper electrophoresis and anion exchange chromatography of the crude reaction product. The lower yields in both cases are probably caused by concurrent oxidation of 3′-hydroxy group to a keto group and subsequent elimination of adenine[28b] which was found to be present in substantial amounts.

(28) (a) A. S. Jones, A. R. Williamson, and M. Winkley, *Carbohydr. Res.*, **1**, 187, (1965); (b) *cf.* A. F. Cook and J. G. Moffatt, *J. Amer. Chem. Soc.*, **89**, 2697 (1967).

to give a solid which was susccessively washed with ether and petroleum ether to give 0.32 g (94%) of **12a**, mp 146–148°. Crystallization from benzene raised the melting point to 148–150°; $[\alpha]^{25}D$ −208.4°, $[\alpha]^{25}_{436}$ −455.8°, $[\alpha]^{25}_{365}$ −792.8°; uv max (95% ethanol) 266 nm (ε 7900), min 234 (ε 1900); nmr ($CDCl_3$) δ 9.73 (broad s, 1, NH), 7.00 (d, 1, H_6), 6.73 (q, 1, $H_{1'}$), 6.43 (q, 1, $H_{4'}$), 5.09 (q, 1, $H_{3'}$), 2.84 (m, 2, $H_{2'}$), 1.90 (d, 3, CH_3). According to tlc (S_2), **12a** was homogeneous.

Anal. Calcd for $C_9H_{10}N_2O_3$: C, 55.66; H, 5.19; N, 14.42. Found: C, 55.79; H, 5.28; N, 14.30.

1-(2′,3′-Dideoxy-3′,4′-didehydro-β-D-erythrofuranosyl)uracil (12b). The same reaction with **1b**[12] was performed as above (6 hr) to give (after crystallization from benzene) 92% of **12b**, mp 142–142.5°: $[\alpha]^{23}D$ −217.4°, $[\alpha]^{23}_{436}$ −479.8°, $[\alpha]^{23}_{365}$ −832.6° (*c* 0.5, 95% ethanol); uv max (95% ethanol) 260 nm, min 230; nmr ($CDCl_3$) δ 7.28 (d, 1, H_6), 6.78 (q, 1, $H_{1'}$), 6.52 (q, 1, $H_{4'}$), 5.81 (d, 1, H_5), 5.19 (q, 1, $H_{3'}$), 2.90 (m, 2, $H_{2'}$).

Anal. Calcd for $C_8H_8N_2O_3$: C, 53.3; H, 4.48; N, 15.55. Found: C, 53.38; H, 4.52; N, 15.30.

1-(2′,3′-Dideoxy-3′,4′-didehydro-β-D-ribofuranosyl)-5-fluorouracil (12c). The reaction with **1c**[13] was performed as above (24 hr). The syrupy residue was partitioned between chloroform and a saturated solution of sodium hydrogen carbonate, and the dried ($MgSO_4$) chloroform layer was evaporated. The residue was treated successively with ether and petroleum ether to give **12c** (25%), mp 169–170°. The aqueous layer was stirred with an excess of Dowex 50 WX 4 (NH_4^+), 100–200 mesh, and the resin was filtered and washed with ethanol. The filtrate was evaporated to afford **12c** (50%), mp 170–173°, which, after crystallization from benzene, showed a melting point of 178–180°: $[\alpha]^{24}D$ −175.2°, $[\alpha]^{24}_{436}$ −378.6°, $[\alpha]^{24}_{365}$ −647.4° (*c* 0.5, $CHCl_3$); uv max (95% ethanol) 268 nm (ε 6550), min 235 (ε 1700); nmr ($CDCl_3$) δ 7.29 (d, 1, H_6), 6.70 (d of q, 1, $H_{1'}$), 6.50 (q partially overlapped with $H_{1'}$, 1, $H_{4'}$), 5.18 (q, 1, $H_{3'}$), 2.92 (m, 2, $H_{2'}$); uniform on tlc (S_2).

Anal. Calcd for $C_8H_7FN_2O_3$: C, 48.49; H, 3.56; N, 14.14. Found: C, 48.76; H, 3.52; N, 13.88.

9-(2′,3′-Dideoxy-3′,4′-didehydro-β-D-erythrofuranosyl)adenine (12d). 2′-Deoxyadenosine-5′-carboxylic acid (**1d**, 0.26 g, 1 mmol) was heated with dimethylformamide dineopentyl acetal (0.69 g, 3 mmol) in dimethylformamide (10 ml) at 80–85° (bath temperature) for 5 hr and the solution was then evaporated to dryness at 50° and 0.1 mm. The residue, on paper electrophoresis, showed no reaction and therefore the whole procedure was repeated,[29] after which paper electrophoresis showed about 80% conversion. Toluene (10 ml) was added, and the reaction mixture was held for 1 hr at 150–160° (bath temperature). Toluene and neopentyl alcohol were removed by distillation, and DMF was evaporated at 0.1 mm and 55°. Paper electrophoresis then showed complete disappearance of the starting material. Uv maxima (95% ethanol) of the residue showed the sole presence of an *N*-dimethylaminomethylene derivative[6] (311 nm, min 249 nm). The syrup was dissolved in methanol (10 ml), 12 *N* ammonium hydroxide (10 ml) was added and the solution was maintained at room temperature for 18 hr. The product **12d**, which crystallized during evaporation, was filtered after addition of ethanol, ether, and petroleum ether, 0.13 g (65%), mp 207–209°, tlc (S_2 and S_3) uniform. After crystallization from ethanol containing a drop of triethylamine, the product showed a melting point of 209–211°; $[\alpha]^{23}D$ −343°, $[\alpha]^{23}_{436}$ −753.8°; $[\alpha]^{23}_{365}$ −1315° (*c* 0.5, 95% ethanol); uv max (95% ethanol) 260 nm (ε 10,600), min 229 (ε 1950); nmr (DMSO-d_6) δ 8.22 (two overlapped s, 2, H_8 and H_2), 7.27 (broad s, 2, NH_2), 6.78 (d of d, 1, $H_{1'}$), 6.63 (q partially overlapped with $H_{1'}$, $H_{4'}$), 5.30 (q, 1, $H_{3'}$), 3.19 (m, 2, $H_{2'}$).

Anal. Calcd for $C_9H_9N_5O$: C, 53.19; H, 4.47; N, 34.47. Found: C, 53.09; H, 4.50; N, 34.24.

Reaction of Thymidine-5′-carboxylic (1a) Acid with Dimethylformamide Dimethyl Acetal (2a). Thymidine-5′-carboxylic acid[9] (**1a**, 1.1 g, 4.27 mmol) was heated with dimethylformamide dimethyl acetal (**2a**, 4 ml, *ca.* 40 mmol) in dimethylformamide (50 ml) for 12 hr at 70°. After cooling, the reaction mixture was evaporated at 0.1 mm and 60°, the syrupy residue was dissolved in chloroform (25 ml), the solution was extracted with water (25 ml), and the extract was dried ($MgSO_4$). Evaporation of the solvent gave, after addition of ether and petroleum ether to the residue, crystalline material (0.6 g) containing, according to tlc (S_2), two major components. The mixture was dissolved in chloroform, applied to three plates of nonadhering (loose-layer) silica gel, and chromatographed in solvent S_1. The two main bands were eluted with solvent S_2 and eluates were evaporated. The slower moving band afforded methyl *N*-methylthymidine-5′-carboxylate (**4**), 0.335 g (29%), tlc S_1 homogeneous, mp 139–141°, after crystallization from methanol mp 150–152°; $[\alpha]^{23}D$ +19.2°, $[\alpha]^{23}_{436}$ +53.6°, $[\alpha]^{23}_{365}$ +121° (*c* 0.5, $CHCl_3$); uv max (95% ethanol) 266 nm (ε 10,500), min 235 (ε 3000); ir (KBr) CO ester, 1760 cm^{-1}; nmr ($CDCl_3$) δ 8.08 (d, 1, H_6), 6.57 (q, 1, $H_{1'}$), 4.58 (s, 2, $H_{3'}$ + $H_{4'}$), 3.80 (s, 3, CH_3O), 3.32 (s, 3, NCH_3), 2.28 (m, 2, $H_{2'}$), 1.98 (d, 3, CH_3-thymine).

Anal. Calcd for $C_{12}H_{16}N_2O_6$: C, 50.70; H, 5.67; N, 9.86. Found: C, 50.70; H, 5.69; N, 10.08.

The faster moving band was worked up as described above to give, after washing the residue with ether and petroleum ether, 0.11 g (17%) of 1,3-dimethylthymine (**8**), mp 143–145°, which, after sublimation at 110° and 0.025 mm, gave 0.07 g (11%) of **8**, mp 153–156°; a mixture melting point with an authentic sample of **8** was undepressed: uv max (95% ethanol) 270 nm (ε 8600), min 238 (ε 2100); nmr ($CDCl_3$) δ 6.99 (d, 1, H_6), 3.42 (s, 3, N_3-CH_3), 3.32 (s, 3, N_1-CH_3), 1.91 (d, 3, CH_3-thymine).

Methyl *N*-Methylthymidine-5′-carboxylate (4). Thymidine-5′-carboxylic acid (**1a**, 0.13 g, 0.5 mmol), suspended in methanol (10 ml), was treated with diazomethane in ether with stirring until a yellow color persisted. The solution was then evaporated and the solid residue was washed with ether–petroleum ether to give 0.125 g (87%) of **4**, mp 137–142°. Crystallization from methanol raised the melting point to 150–153°, which was undepressed on admixture with a sample of **8** obtained as described above; uv, ir, and nmr spectra of the samples were identical.

1,3-Dimethylthymine (8). Thymine (0.252 g, 2 mmol) was heated with dimethylformamide dimethyl acetal (2 ml, *ca.* 20 mmol) in dimethylformamide (10 ml) for 7.5 hr at 80–90° (bath temperature). After 5.5 hr, tlc (S_2) showed a quantitative conversion of thymine to **8**. The solution was evaporated at 0.1 mm and 40° to a solid which sublimed at 80–90° and 0.1 mm to give 0.285 g (92%) of **8**, mp 153–156° (lit.[30] 151–153°). Nmr, ir, and uv spectra were identical with those of compound **8** above.

1-(2′,3′-Dideoxy-β-D-erythrofuranosyl)thymine (13a). Compound **12a** (100 mg, 0.55 mmol) was hydrogenated over 10% palladium on charcoal (100 mg) in ethanol (10 ml) in Brown's apparatus[31] for 2.5 hr at room temperature. The catalyst was filtered off, the solution was washed first with ethanol and then acetone, and the filtrate was evaporated to dryness. The syrupy residue was dissolved in saturated sodium bicarbonate (5 ml) and extracted twice with chloroform (10 ml), and the extract ($MgSO_4$) was evaporated to a solid which was collected after addition of petroleum ether, 0.08 g (79%), mp 134–136°, unchanged after recrystallization from benzene. The analytical sample was dried at 10^{-3} mm and 100°: $[\alpha]^{27}D$ +36.8°, $[\alpha]^{27}_{436}$ +112°, $[\alpha]^{27}_{365}$ +269.4° (*c* 0.5, $CHCl_3$); uv max (95% ethanol) 268 nm (ε 9600), min 235 (ε 1900); nmr ($CDCl_3$) δ 7.11 (d, 1, H_6), 6.00 (q, 1, $H_{1'}$), 4.07 (q, 2, $H_{4'}$), *ca.* 2.09 (m, 4, $H_{2'}$ + $H_{3'}$ partially overlapped with CH_3), 1.92 (d, 3, CH_3).

Anal. Calcd for $C_9H_{12}N_2O_3$: C, 55.09; H, 6.17; N, 14.28. Found: C, 54.86; H, 6.14; N, 14.12.

1-(2′,3′-Dideoxy-β-D-erythrofuranosyl)-5-fluorouracil (13c). Compound **12c** (0.25 g, 1.25 mmol) was hydrogenated over 0.25 g of Pd/C as described above. The catalyst was filtered and washed with ethanol, and the filtrate was evaporated to dryness. The solid residue was washed with petroleum ether to give **13c** (0.2 g, 80%), homogeneous on tlc (S_2), mp 165–167°. Recrystallization from benzene gave a crystalline solid, mp 174–176°; $[\alpha]^{23}D$ +56°, $[\alpha]^{23}_{436}$ +158.4°, $[\alpha]^{23}_{365}$ +359.2° (*c* 0.5, $CHCl_3$); uv max (95% ethanol) 270 nm (ε 7200), min 235 (ε 1400); nmr ($CDCl_3$) δ 7.43 (d, 1, H_6), 6.00 (poorly resolved m, 1, $H_{1'}$), 4.10 (m, 2, $H_{3'}$), 2.12 (m, 4, $H_{2'}$ + $H_{3'}$).

Anal. Calcd for $C_9H_9FN_2O_3$: C, 48.00; H, 4.53; N, 14.00. Found: C, 47.72; H, 4.59; N, 13.71.

9-(2′,3′-Dideoxy-β-D-erythrofuranosyl)adenine (13d). Compound **12d** was hydrogenated as described above (0.3 g, 1.48 mmol)

(29) The lack of reaction under the conditions currently used for decarboxylative elimination of uronic acids **1a–c** may be explained by a shift in equilibrium (**1** ⇄ **10**) caused by an increased amount of neopentyl alcohol in the reaction mixture. Formation of *N*-dimethylaminomethylene derivative[6] in this case gives rise to an additional 2 mol of neopentyl alcohol, while in the formation of **10** and **12** also 2 mol of neopentyl alcohol are released.

(30) E. Wittenburg, *Chem. Ber.*, **99**, 2380 (1966).

(31) C. A. Brown and H. C. Brown, *J. Amer. Chem. Soc.*, **84**, 2829 (1962).

in ethanol–dioxane (2:1) mixture (60 ml) over Pd/C (0.3 g) and the reaction mixture was worked up as described for **13c**. The syrupy product gave, after stirring with ether, a solid **13d** (0.24 g, 79%), homogeneous on tlc (S_2 and S_3), mp 140–142°. Crystallization of the product from ethanol raised the melting point to 150–151°, which was not depressed on admixture with a sample of the racemic compound (lit.[25] mp 165–168°): $[\alpha]^{25}D$ −33.4°, $[\alpha]^{25}_{436}$ −74.4°, $[\alpha]^{25}_{365}$ −136.6° (*c* 0.5, $CHCl_3$); uv max (95% ethanol) 260 nm, min 227; nmr ($CDCl_3$) δ 8.33 (s, 1, H_8), 7.92 (s, 1, H_2), 6.31 (t, 1, $H_{1'}$ partially overlapped with NH_2), 6.43 (broad s, 2, NH_2), 4.14 (q, 2, $H_{4'}$), *ca.* 2.31 (m, 4, $H_{2'} + H_{3'}$). Uv, ir, and nmr spectra correspond to those of the racemic product.

Anal. Calcd for $C_9H_{11}N_5O$: C, 52.68; H, 5.40; N, 34.13. Found: C, 52.47; H, 5.42; N, 33.92.

3-*N*-Methyl-5-fluoro-2′-deoxyuridine (9b).[32] A solution of 5-fluoro-2′-deoxyuridine (**9a**, 0.25 g, 1 mmol) and dimethylformamide dimethyl acetal (1 ml, *ca.* 10 mmol) in dimethylformamide (10 ml) was heated 5.5 hr at 90° (bath temperature) and the reaction mixture was evaporated to a syrup (0.1 mm, 50°). The addition of ether together with chilling to −20° produced a crystalline solid, 0.21 g (81% yield) of **9b**, mp 75–80°, which was homogeneous on tlc (S_2). The analytical sample was crystallized from ethyl acetate (0.15 g, 58%), mp 80–81° (sinters from 60°): $[\alpha]^{22}D$ +33.2°, $[\alpha]^{22}_{436}$ +89.6°, $[\alpha]^{22}_{365}$ +200.8° (*c* 0.5, dioxane); uv max (95% ethanol) 269 nm (ε 7400), min 236 (1100); nmr (CD_3COCD_3) δ 8.24 (d, 1, H_6), 6.32 (t, 1, $H_{1'}$), 4.50 and 3.97 (m, 2, $H_{4'} + H_{3'}$), 3.31 (s, 3, NCH_3), 2.35 (m, 2, $H_{2'}$), 2.89 (broad s, 2, OH), 3.84 (d, 2, $H_{5'}$).

Anal. Calcd for $C_{10}H_{13}N_2FO_5 \cdot H_2O$: C, 43.17; H, 5.41; N, 10.07. Found: C, 43.37; H, 5.50; N, 9.92.

(32) This compound was mentioned briefly by T. A. Khwaja and C. Heidelberger, *J. Med. Chem.*, **13**, 64 (1970), without providing details of the method of preparation and without characterization.

Acknowledgments. The authors are indebted to Mr. N. Cvetkov for measuring uv, ir, and nmr spectra and optical rotations, and also to the Cancer Chemotherapy National Service Center, National Cancer Institute, Bethesda, Md., for providing us with a sample of racemic **13d**. The authors gratefully acknowledge the generous sample of 5-fluoro-2′-deoxyuridine provided by Hoffmann-La Roche Co., Nutley, N. J.

Tautomerism of Nucleic Acid Bases. II. Guanine[1]

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Abstract: It has long been noted that the H_8 proton of guanosine and its related derivatives exhibits unusually broad resonances in the pmr spectra under certain conditions of temperature and pD. We have examined this phenomenon as a function of temperature, concentration, and solution pD, as well as the external magnetic field, and have shown that the line broadening arises from chemical exchange between the lactam and lactim tautomers of the guanine base. The observation of sharp H_8 resonances in guanine derivatives where the guanine base is frozen in only the lactam and lactim tautomeric structure supports this interpretation. This tautomeric exchange was found to be catalyzed by OD^- and the solvent D_2O. Analysis of the temperature and pD dependence of the H_8 line width for 2′-GMP led to the following rate law at 30°: $-(d[A]/dt)_f = 1.7 \times 10^9[OD^-][A] + 0.7[D_2O][A]$ M sec^{-1}, where A represents the major tautomeric species of 2′-GMP. Activation energies of 7 and 13 kcal/mol were deduced for the OD^-- and D_2O-catalyzed steps, respectively. The minor lactim tautomer was estimated to be present to the extent of 16 ± 3% at room temperature in neutral aqueous solution. In most guanosine derivatives, with the notable exception of 2′-GMP, the H_8 line width was also found to be strongly concentration dependent over the pD range 3–6. This concentration dependence has been interpreted in terms of the effect of intermolecular association on the lactam–lactim tautomeric equilibrium. Analysis of the data in terms of a rapid equilibrium involving the monomer, a hydrogen-bonded tetramer, and stacked aggregates of this tetramer yielded a tetramer formation constant of 2.5 ± 0.5 × 10^7 M^{-3} and a tetramer stacking or association constant of 40 ± 10 M^{-1} in the case of 5′-GMP.

In a recent paper,[2,3] we showed that the unusual broadening of the cytosine H_5 resonance frequently observed in the pmr spectrum of cytosine and its nucleoside and nucleotide derivatives is due to tautomeric exchange between the amino and imino tautomers of the cytosine base. A detailed study of this line-broadening phenomenon has been made as a function of temperature, concentration, solution pH, and external magnetic field, and quantitative treatment of the linewidth data enabled us to ascertain both the equilibrium and the kinetics of this tautomerism. Contrary to what has generally been accepted,[4–6] the cytosine base was found to exist in a significantly high percentage in the abnormal tautomer (15 ± 3%) at room temperature. The kinetics data revealed both a solvent- and base-catalyzed step, but the rate of the tautomeric exchange was found to be quite slow. However, this slow kinetics is quite reasonable if the rate-determining step involves proton abstraction from the cytosine amino group by base or a solvent molecule.

This paper concerns a similar study with the guanine base. It has long been noted that the H_8 proton of guanosine and its derivatives also exhibits an unusually

(1) This work was supported in part by Grants GM 14523-04 and -05 from the National Institute of General Medical Sciences, U. S. Public Health Service, and Grant No. GP-8540 from the National Science Foundation.

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